Grandlure Dosage and Attraction of Boll Weevils (Coleoptera: Curculionidae)

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ABSTRACT The effects of grandlure dosage on of boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), attraction were assessed. Traps collected more boll weevils under field and laboratory conditions as the amount of grandlure in laminated plastic strips was increased from 0 to 10, 30, and 60 mg. Spreading the point source of the lure by cutting the strip into quarters and positioning each quarter on separate corners of the large capacity trap to create an expanded source for the pheromone plume, however, resulted in fewer trap captures than traps with quartered lures all positioned on a single corner. The large capacity trap with the quartered lure on one corner also caught more weevils than the traps with an intact lure fastened to one corner. Although aging lure strips for three weeks reduced emissions of the four pheromone components and their attractiveness to boll weevils, cutting the aged lure into quarters resulted in greater emissions and attraction than lures that were aged intact or as quarters. Some pheromone components volatilized faster than others, resulting in time-related changes in blend ratios, but the underlying factor in boll weevil attraction to grandlure strips was dosage, the amount of volatilized pheromone available for interacting with an adult boll weevil.

KEY WORDS *Anthonomus grandis*, boll weevil, grandlure, pheromone, trapping

The boll weevil, *Anthonomus grandis grandis* Boheman (Coleoptera: Curculionidae), is originally from the tropics and subtropics of Mesoamerica (Burke et al. 1986), but the present-day distribution extends from temperate parts of the U.S. Cotton Belt to Argentina (Rummel and Summy 1997, Ramalho and Jesus 1988, Cuadrado 2002). Boll weevil trapping has an important role in control strategies, especially the eradication programs that have occurred in the United States (Dickerson et al. 2001). The most commonly used boll weevil attraction pheromone-based lure for eradication and suppression programs is Hercon (Hercon Environmental, Emigsville, PA) grandlure (Tumlinson et al. 1969, Rummel et al. 1980, Dickerson 1986) (10 mg) impregnated in 22- by 27-mm plastic laminate strips (Quisumbing and Kydonieus 1982). Although the lure is mostly used for boll weevil monitoring (McKibben et al. 2001), researchers also use it for studying field populations and for collecting live specimens (Guerra et al. 1982, Showler 2003).

Increasing the grandlure dosage emitted by wick and cigarette filter (McKibben et al. 1980) dispensers, and slow release formulations (Leggett and Taft 1979), in traps increased boll weevil captures (Johnson et al. 1982, Leggett 1982). Greater amounts of alcohols also significantly improved the performance of grandlure (Hardee et al. 1974). Although several grandlure dispensers and formulations have been assessed as well as different amounts of grandlure, the effects of different amounts in the plastic laminate dispenser on the response of boll weevils has not been determined.

A variety of factors affect the attractiveness of pheromone-based lures, including dose, release rate, blend of active volatiles, and dispensers (Hardee et al. 1974; Pivnick et al. 1988; Jansson et al. 1990, 1992, 1993; Mason et al. 1990; Evenden et al. 1995; López 1998; Showler et al. 2005). Temperature can influence release rates of various pheromone lures to different extents. For example, grandlure release rates were found to increase by as much as 13-fold as the temperature was raised from 28 to 62°C (Leonhardt et al. 1988), and gypsy moth, *Lymantria dispar* (L.), (+)-disparlure release rates from plastic laminate dispensers increased 2.5-fold for every increase of 10°C (Leonhardt et al. 1990). Leonhardt et al. (1988) "accelerated aging" of grandlure by increasing temperature, and this resulted in reduced emissions from the "temperature-aged" lure. Aging, without intentional heat acceleration, of pheromone-based lures results in a decline in insect response (Mason et al. 1990, López 1998, Showler et al. 2005), but the rate of response can vary substantially. For example, after 1 wk of aging

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pheromone-containing rubber septa, responses of nitidulid beetles species ranged from 18% of its original value to 90% (Bartelt et al. 1994).

Boll weevil eradication efforts (Dickerson et al. 2001) are expected to resume in the subtropical Lower Rio Grande Valley of Texas, and boll weevil monitoring will continue in the subtropical and tropical cotton-growing areas south of the Rio Grande to Argentina. The objective of this study was to evaluate the effects of pheromone dosage emitted from grandlure strips on collecting boll weevils in traps under the subtropical and tropical conditions that make up most of the boll weevil's distribution.

Materials and Methods

Grandlure Dosage. Two experiments were conducted to assess the effect of different amounts of grandlure in laminate strips on attraction of boll weevils. Large capacity boll weevil traps were used because they are better than the conventional Hercon trap (Hercon Environmental) for measuring moderate and high numbers of weevils responding to the lure (Showler 2003). One experiment occurred in Hidalgo County, TX, and the other occurred 7 km east of Gonzales, Tamaulipas, Mexico. Treatments at both locations were 10, 30, and 60 mg of grandlure (using 10-mg lure strips only), and a control trap with no grandlure. The four treatments were spaced 35 m apart in a line along one edge of a commercial cotton field after routine cotton harvest and stalk destruction operations were completed, when the numbers of boll weevils responding to grandlure are high near cotton fields relative to other times of the year (Parajulee and Slosser 2001, Showler 2003). The numbers of captured boll weevils were counted every 48 h, 14–30 July 2001, in Texas and every week, 28 November 2000–23 January 2001, in Tamaulipas. Pheromone lures were changed with new lures weekly. Trap positions were randomly reassigned within each replicate of traps at each sampling interval to minimize the possibility of position effects. In Texas and Tamaulipas, there were five and six replicates, respectively, and each replicate was deployed along a separate cotton field.

The three amounts of grandlure and a control (no lure) were compared in a controlled environment by placing each in a separate Hercon trap mounted on a 1-m-tall pole. Each Hercon trap was deployed in a separate 1.5- by 2.5- by 2.5-m (width by length by height) cage in the laboratory ($n = 10$ replicates) at 29.4°C and 25% RH. Fifty randomly selected field-collected boll weevils (sex ratios not determined) were released in each cage, and trap captures were recorded 1.5 h later.

In the field experiments, treatment and time effects on numbers of collected boll weevils, and treatment by time interaction, were detected with repeated measures analyses (Analytical Software 1998). Treatment differences in the cage assays were detected using one-way analysis of variance (ANOVA), and means were separated using Tukey's honestly significant difference (HSD) (Analytical Software 1998).

Aging and Cutting Grandlure Strips. Two field experiments to evaluate the effects of aging and cutting grandlure strips on trapping efficiency were conducted in Hidalgo County, one experiment 8–17 July 2001 ($n = 7$ replicates per treatment) and the other experiment 14–23 August 2002 ($n = 5$ replicates per treatment). In one of the five treatments, a 22- by 27-mm 10-mg grandlure strip was cut with scissors into four approximately equal quarters, all skewered through their centers, 0.5 cm apart, on a 5-cm-long sewing needle, and placed together in a Hercon trap. In the second treatment, the needle with the skewered lure quarters was fastened with a clamp to one upper corner of a large capacity boll weevil trap. In the third treatment, the quarters were each fastened to a separate corner of a large capacity trap, and the fourth treatment consisted of a large capacity trap with an uncut (intact) lure attached to an upper corner. A large capacity trap with no lure served as the control. The width by height of the large capacity trap was 22.8 by 122 cm.

Each set of treatments, including the control, was deployed in a line, traps 35 m apart, along one edge of a separate commercial cotton field. Boll weevils collected in the traps were counted, and the positions of the traps were rerandomized within each trap line every 48 h. The lures were replaced with fresh lures after 7 d. Treatment effects were detected using one-way ANOVA, and means were separated using Tukey's HSD (Analytical Software 1998).

The attractivity of five cutting and aging treatment combinations on 10-mg grandlure strips to adult boll weevils under controlled conditions were assessed with a control (no lure) ($n = 10$ replicates per treatment). Hercon traps were used because the possibility of passive collection of weevils is likely lower than for large capacity traps (Showler 2003), especially in a cage. Intact lures and lures cut into quarters were either used immediately after opening the sealed refrigerated (0°C) packets containing the lures or after 3 wk of aging the strips in sunlight during summer 2004 at ≈35°C during the day and 35% RH in Harlingen, Cameron County, TX. Lures that were aged intact and then cut into quarters comprised the fifth treatment. The Hercon traps were placed individually in the cages. Fifty randomly selected field-collected adult boll weevils (sex ratios not determined) were released in each cage and trap captures were recorded 1.5 h later.

The same five treatments, aged outdoors in full sun during July 2001, Hidalgo County, were analyzed for volatile emissions of the four key attractive components of grandlure: (1*R*,2*S*)-1-methyl-2-(1-methylethenyl) cyclobutaneethanol, (2*Z*)-2-(3,3-dimethylcyclohexylidene) ethanol, *cis*-3,3-dimethylcyclohexylidene acetaldehyde, and *trans*-3,3-dimethylcyclohexylidene acetaldehyde ($n = 9$) (Bull et al. 1971, Dickens et al. 1991), hereafter referred to as A, B, C, and D, respectively. The lures and quarters were placed inside a 20-ml vial where they were leaned against the side of the vial standing on end (each quarter occupied a different quadrant). Vola-

tiles were sampled using solid phase microextraction (Bartelt 1997) with a polydimethylsiloxane (PDMS)-coated (100-μm coating) fiber (Supelco, Bellefonte, PA). The fiber was inserted into the vial through a hole in the vial cap with the same diameter as the fiber to prevent leakage. The tip of the fiber was 7 mm above the floor of the vial at a level below the center of whole lures and slightly above the centers of quarters. Sampling time was 1 h at 30°C.

Volatilized components of the attractant pheromone were quantified using a Shimadzu gas chromatograph-17A (Shimadzu Scientific Instruments, Columbia, MD) with a flame ionization detector. On-column injection of volatiles was accomplished by thermal desorption from the PDMS fiber at 220°C in a 15-cm retention gap (0.53 mm i.d. deactivated fused-silica) connected to the analytical column by a GlasSeal connector (Supelco). The analytical column was a DB-1 (60 m, 0.32 mm i.d., 5-μm film) (J & W Scientific, Folsom, CA). Analyses were conducted at a column oven temperature of 160°C. Carrier gas was helium at a linear velocity of 30 cm/s. Chromatographic peak areas were integrated using Millenium 2010 Chromatography Manager (Waters, Milford, MA) software.

Before quantification of emissions from lures could be conducted, it was necessary to determine identities of the eluted peaks. Identification of lure components was done by gas chromatography–mass spectrometry (GC-MS) with an Agilent 6890 series GC (Agilent Technologies, Atlanta, GA) with an Agilent 5973 network mass selective detector (EI) (electron energy = 70 eV) operated over a mass range of 40–550 amu. The system was controlled by an Agilent MS Chemstation. Volatiles were collected by solid phase microextraction by inserting the fiber briefly into the headspace of a vial containing a grandlure standard (obtained from USDA–ARS, College Station, TX). Chemicals were thermally desorbed from the fiber in a split/splitless injector in the splitless mode at 250°C. The analytical column for GC-MS was the same DB-1 column described above. Linear velocity of helium carrier gas as 40 cm/s. Column oven temperature was programmed from 50 to 160°C at 5°C/min and holding the final temperature for 50 min. GC-MS identifications were based on computer matching of unknown spectra with those in the NIST 98 Library of Mass Spectra and Subsets (Agilent Technologies). Volatile blend ratios were calculated by comparing the amount (measured as peak areas, expressed as milli-electron volts [MeV]) of each volatile emitted against the others. Treatment effects were detected using one-way ANOVA, and means were separated using Tukey's HSD (Analytical Software 1998). Ratios were arcinse-square root-transformed before ANOVA, but nontransformed data are presented.

Results

Grandlure Dosage. Repeated measures analysis of the Texas data detected treatment ($F = 9.21$; df = 3, 160; $P \leq 0.0001$) effects. Mean numbers of captured

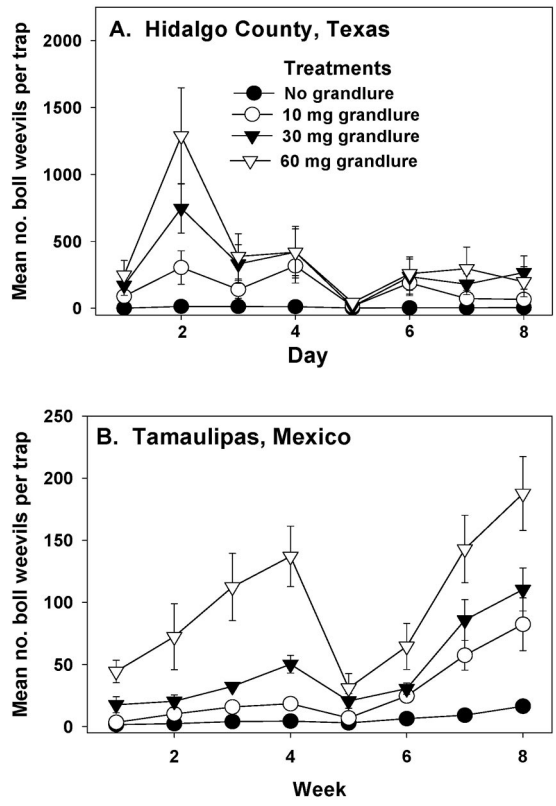

Fig. 1. Mean ± SE numbers of boll weevils collected at weekly intervals on large capacity traps with 10-, 30-, and 60-mg grandlure strips, 14–28 July 2001, Hidalgo County, TX ($n = 5$) (A), and 28 November 2000–23 January 2001, Tamaulipas, Mexico ($n = 6$) (B).

weevils in the treatments were greater than in the control when weevil populations responding to the pheromone were highest, principally during the second week of sampling (Fig. 1A), but differences were less apparent as populations declined. Treatment ($F = 52.54$; df = 3, 128; $P \leq 0.0001$) effects also were found in Tamaulipas. Mean numbers of boll weevils collected in the traps were consistently greatest in the traps with the 60-mg lures, intermediate in the 10- and 30-mg lure traps, and lowest in the control traps (Fig. 1B). This pattern was apparent when trap captures were high or low, but significant treatment differences were most apparent when populations of weevils responding to the lures were high.

Differences were detected between the amounts of grandlure used in the cage assay ($F = 345.37$; df = 3, 39; $P < 0.0001$). Each successively higher amount of grandlure collected 46 and 38% more ($P < 0.05$) weevils than the amount before it (Fig. 2).

Aging and Cutting Grandlure Strips. A significant treatment effect was detected for mean numbers of boll weevils caught in the 2001 treatment traps ($F = 99.12$; df = 4, 34; $P \leq 0.0001$). The large capacity trap with the skewered lure quarters caught 6.6, 1.5, and 1.9

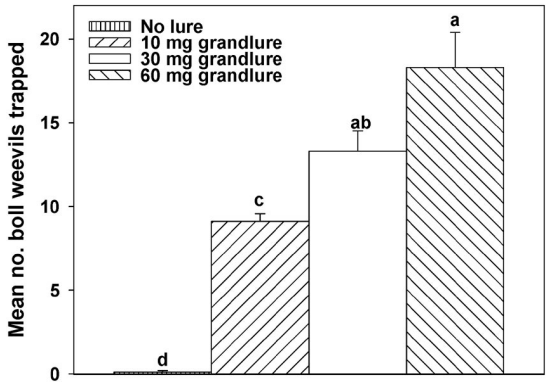

Fig. 2. Mean ± SE numbers of boll weevils collected in Hercon traps with different dosages of grandlure, laboratory cage assay ($n = 10$).

times more weevils than the Hercon trap, and the large capacity traps with the whole and spread lures, respectively ($P \leq 0.05$) (Fig. 3A). In the 2002 experiment, a treatment effect also was detected ($F = 10.24$; df = 4, 24; $P = 0.0005$). The large capacity trap with the skewered lure quarters caught 5.2, 1.6, and 1.7 times ($P \leq 0.05$) more weevils than the Hercon trap, and the large capacity traps with the whole and spread lures, respectively (Fig. 3B).

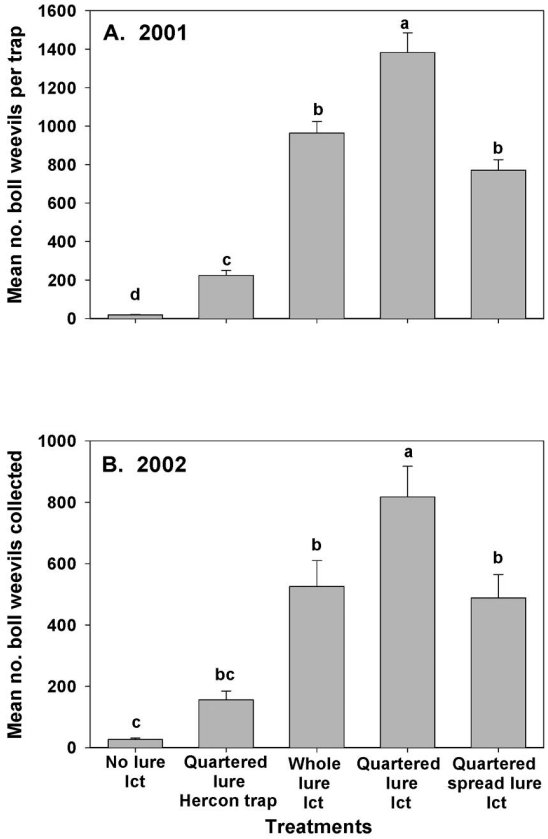

Fig. 3. Mean ± SE numbers of boll weevils collected in large capacity traps (lct) that were attracted by plastic grandlure strips cut into quarter sections and skewered on a single needle or spread (one quarter on each corner of the trap), presented intact, or quartered and placed in a Hercon traps, 8–17 July 2001 ($n = 7$) (A) and 14–23 August 2002 ($n = 5$), Hidalgo County, TX (B).

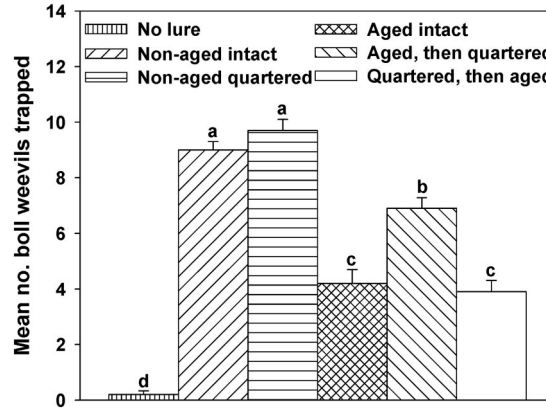

Fig. 4. Mean ± SE numbers of boll weevils collected in Hercon traps with grandlure strips that were treated with different combinations of aging for 3 wk at 32.2°C and cutting the lure into quarters, cage assay ($n = 10$).

In the cage assay, differences in mean numbers of trapped boll weevils were found ($F = 89.73$; df = 5, 59; $P < 0.0001$) between treatments (Fig. 4). The lures that were aged before being quartered collected 41 and 30% more weevils than non-aged intact and quartered lures, respectively ($P < 0.05$). The lures that were aged then quartered were 64 and 77% more efficient than the aged intact and aged quartered lures ($P < 0.05$), respectively, and each of those treatments collected more weevils than the control ($P < 0.05$) (Fig. 4).

Differences were detected between the five treatments after solid phase microextraction for each of the four key components of the grandlure pheromone ($10.41 \leq F \leq 46.17$; df = 4, 44; $P < 0.0001$). The non-aged intact and quartered lures emitted 1.9- to 5-fold more A and C ($P < 0.05$) than any of the aged treatments, and the non-aged quartered lure emitted 1.6- to 2.4-fold more B and D ($P < 0.05$) (Fig. 5A–D). The lures that were aged, then quartered, produced 1.5- to 2-fold more A, C, and D ($P < 0.05$) than the aged quarters or intact lures (Fig. 5A, C, and D).

Treatment effects were detected for blend ratios C:A ($F = 11.85$; df = 4, 44; $P < 0.0001$), C:B ($F = 3.16$; df = 4, 44; $P = 0.0239$), and D:C ($F = 5.29$; df = 4, 44; $P = 0.0016$). Cutting the lure, whether aged or not, was associated with greater ratios of B:A than intact lures (Table 1). The ratios of C:A were greatest ($P < 0.05$) for lures that were either not aged or freshly quartered (Table 1). The ratios of C:B were greatest for non-aged lures ($P < 0.05$), but C:D was greatest in the aged treatments ($P < 0.05$) (Table 1). Ratios of B:A, D:A, and D:B were not affected by the treatments.

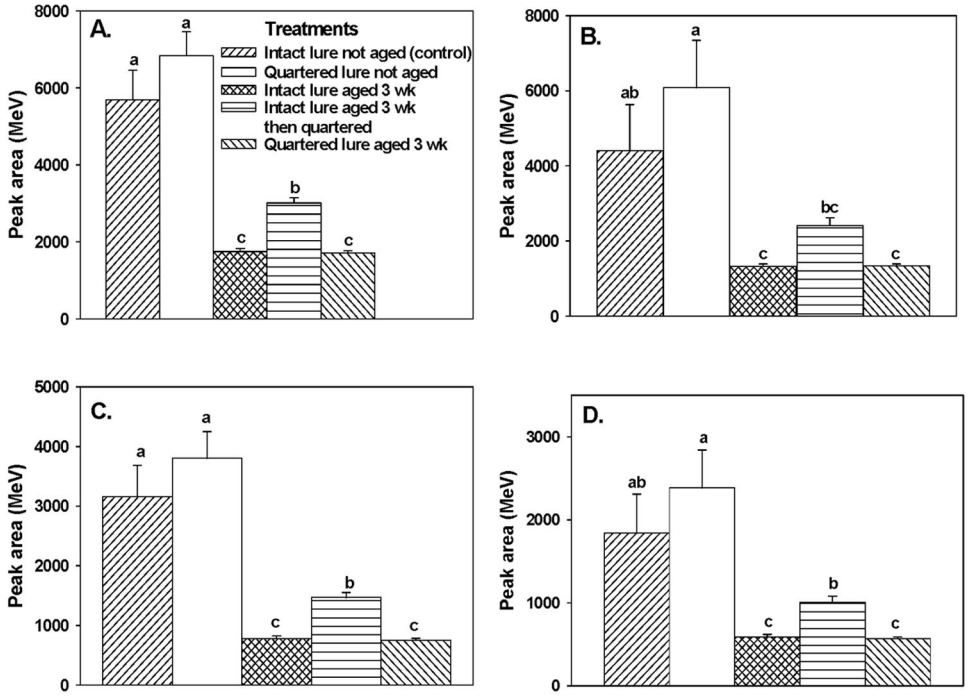

Fig. 5. Mean ± SE MeV during solid phase microextraction of (A) (1*R*,2*S*)-1-methyl-2-(1-methylethenyl) cyclobutaneethanol, (B) (2Z)-2-(3,3-dimethylcyclohexylidene) ethanol, (C) *cis*-3,3-dimethylcyclohexylidene acetaldehyde, and (D) *trans*-3,3-dimethylcyclohexylidene acetaldehyde components of grandlure pheromone strips treated with different combinations of aging for 3 wk at 32.2°C and cutting the lure into quarters.

Discussion

The field assays in Texas and Tamaulipas demonstrate that laminate strips with larger amounts of grandlure attract more adult boll weevils, particularly when populations that are responding to the pheromone are moderate or high. This agrees with other studies where different dispensers were used (Leggett and Taft 1979, McKibben et al. 1980, Johnson et al. 1982). Our findings also show that the large capacity boll weevil trap is more efficient for moderate and high adult boll weevil populations than the Hercon trap (Showler 2003). Relatively low variation in the cages permitted separation of means between each treatment mean and the control and showed an association between the amounts of grandlure in the dispenser and boll weevil response.

The 2001 and 2002 grandlure strip aging and cutting field experiments showed that when four quarters of a 10-mg grandlure strip were kept together on the large capacity trap, more boll weevils were attracted than to traps with whole or quartered but spread lures. Volatile pheromone emission from the trap can be

Table 1. Mean ± SE ratios of the four key volatile components of laminate plastic grandlure strips in aging and cutting treatments

Ratio[a]	Non-aged intact[b]	Non-aged quartered[c]	Aged intact[d]	Aged intact then quartered[e]	Quartered then aged[f]
B:A	0.66 ± 0.10	0.75 ± 0.10	0.76 ± 0.004	0.80 ± 0.05	0.72 ± 0.004
C:A	0.53 ± 0.02a	0.54 ± 0.02a	0.44 ± 0.01c	0.48 ± 0.007bc	0.44 ± 0.005c
D:A	0.30 ± 0.04	0.32 ± 0.04	0.33 ± 0.006	0.33 ± 0.02	0.33 ± 0.003
C:B	0.79 ± 0.08a	0.74 ± 0.08a	0.58 ± 0.01b	0.62 ± 0.008b	0.56 ± 0.006b
D:B	0.44 ± 0.02	0.41 ± 0.01	0.44 ± 0.008	0.42 ± 0.01	0.42 ± 0.004
D:C	0.55 ± 0.06b	0.57 ± 0.06b	0.76 ± 0.003a	0.69 ± 0.04ab	0.75 ± 0.006a

Means followed by different letters in the same row are significantly different ($P < 0.05$), one-way ANOVA, Tukey's HSD. Ratios were arcsine-square root-transformed before ANOVA, but nontransformed ratios are presented.

[a] A, (1*R*,2*S*)-1-methyl-2-(1-methylethenyl cyclobutaneethanol; B, (2Z)-2-(3,3-dimethylcyclohexylidene) ethanol; C, *cis*-3,3-dimethylcyclohexylidene acetaldehyde; and D, *trans*-3,3-dimethylcyclohexylidene acetaldehyde.

[b] Control.

[c] Strip was cut into quarters and immediately analyzed for volatiles.

[d] Aged intact for 3 wk and analyzed for volatiles.

[e] Strip was aged intact for 3 wk and then quartered and analyzed for volatiles.

[f] Strip was cut into quarters, and then the quarters were aged for 3 wk and analyzed for volatiles.

increased by using more than one 10-mg grandlure strip, by using strips with greater amounts of grandlure, or by cutting the strip into pieces. Spreading the quarters on the large capacity trap probably resulted in dilution and faster attenuation of the pheromone plume, reducing its capacity to attract boll weevils downwind.

The reduced efficiency of the quartered lure in the Hercon trap compared with the large capacity trap most likely resulted from difference in trap design affecting weevil access to the trap itself (Showler 2003). It is conceivable that trap design also might have a mitigating effect on delivery of the pheromone into the environment. The Hercon trap ostensibly dispenses the volatile pheromone from a perforated 5-cm-diameter plastic cap, but there are also holes that an allow air and volatiles to pass freely, diluting the pheromone at its source.

In the cage assay, treatment effects on boll weevil trap captures reflected the trends observed for amounts of each pheromone component emitted from the lure strips. The assay demonstrated that aging lures for 3 wk under subtropical conditions has a negative effect on boll weevil response. However, we also showed that fresh exposure of some surface area had a positive effect on the amounts of volatilized A, C, and D, and on boll weevil response in the cage assay. Cutting the lures exposes fresh surface area from which the impregnated pheromone can volatilize.

Smaller C:A and C:B ratios were associated with aged lures, indicating that C (an aldehyde) is more volatile than A and B (alcohols). The lower D:C ratio for non-aged lures indicates that C also volatilizes more than D. Gradual loss of the four grandlure components over time was associated with declines in boll weevils collected in the cage assay. Although both amounts of the pheromone components being emitted, and their blend ratios, changed with aging, we were unable to determine which, if any, plays the most important role in eliciting adult boll weevil response.

In the context of pheromones, the term "dosage" should refer to the quantity of pheromone that interacts with the receiving insect. For inducing insect attraction, the amount of pheromone emission from the lure is more important than the amount retained in the lure, and the consistency, or blend, or the retained amount can change over time. Because aging and cutting lures each affect pheromone emissions from the lure, they affect dosage.

Laminate grandlure dispensers emit less of all four volatile grandlure components and attract fewer boll weevils when the lure has been aged for 3 wk in the subtropics. The loss in the dispenser's capacity to attract boll weevils after aging indicates a loss in effectiveness; also, because some adjustment to trap capture interpretation becomes necessary, the lure is less efficient for monitoring purposes. However, the observed increases in three of the four key grandlure constituents in cut aged laminate dispensers suggest that field-aged lures might be reusable if sufficient fresh surface area can be exposed.

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